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# Prevalence of hepatitis A virus in sea food in Iran

[*Prevalência de vírus da hepatite A em frutos do mar no Irã*]

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## ABSTRACT

The objective of this study was to determine the prevalence of Hepatitis A Virus (HAV) in sea food samples in the Isfahan and Shahrekord townships in Iran. From September 2010 to April 2011, a total of 300 samples of fresh fish, shrimp, crab and lobster were obtained from randomly selected retail stores in the Isfahan and Shahrekord townships in Iran. The samples were tested for the presence of HAV using a reverse transcriptase- polymerase chain reaction method. Out of the total number of samples examined, 8 (2.7%) were found to be positive for HAV. This virus was detected in 5% and 1.7% of fresh fish and shrimp, respectively. This study shows the importance of sea food as potential sources of HAV infection in people in Iran.

Keywords: HAV, sea food, RT-PCR, Iran

## RESUMO

O objetivo deste estudo foi determinar a prevalência do vírus Hepatitis A (HAV) em amostras de frutos do mar nas cidades de Isfahan e Shahrekord no Iran. De setembro de 2010 a Abril de 2011 um total de 300 amostras de peixe fresco, camarão, caranguejo e lagosta foram obtidas de lojas de varejo aleatoriamente escolhidas nas cidades de Isfahan e Shahrekord no Iran. As amostras foram testadas para presença de HAV usando o método de reação em cadeia em transcriptase reversa. Do total de amostras examinadas, 8 (2.7%) foram positivas para HAV. Este vírus foi detectado em 5% e 1.7% de peixe fresco e camarão, respectivamente. Este estudo mostrou a importância de frutos do mar como fontes potenciais de infecção HAV em pessoas no Iran.

Palavras chave: HAV, frutos do mar, RT-PCR, Iran

## INTRODUCTION

In developed countries, foodborne or waterborne hepatitis A (HA) outbreaks are relatively uncommon (Acheson and Fiore, 2004). However, infected food handlers remain the source of most reported foodborne outbreaks (Fiore, 2004). In many low endemicity countries, the potential for food contamination from an infected food handler is a recognized public health concern (Koopmans *et al*., 2003). In these countries, a large proportion of the population has never been exposed or vaccinated against hepatitis A virus (HAV) and is thus susceptible to infection during potential outbreaks (Scheifele, 2005; Tricco *et al*., 2006).

HAV may cause hepatitis 2–6 weeks after exposure. Infection and vaccination generally result in long-term immunity. In children, HAV infection is often asymptomatic. Symptoms of infection are more severe in older adults, with a case fatality rate of 0.8% in people aged >40 years of age (Brown and Persley, 2002). Transmission follows the fecal-oral route, and mainly occurs through contact with symptomatic or asymptomatic infected persons, i.e. person-to-

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person transmission. HAV has recently been recognized as a sexually transmitted infection, especially in men who have sex with men (MSM) (Urbanus *et al*., 2002).

Infection may also be foodborne, i.e. after ingestion of contaminated food. Underreporting of outbreaks due to foodborne sources is likely, as patients need to recall their exposures of 2–6 weeks preceding their illness. HAV is endemic in most countries in Africa, Asia, South America and Central America (Jacobsen and Koopman, 2004; Verhoef *et al*., 2011). For most Western countries such as the USA, Australia and countries in Europe, the risk of HAV outbreaks is changing because endemic circulation has become less common with the improvement of sanitary conditions. Consequently, the non-vaccinated population has become more susceptible (Koopmans *et al*., 2003).

Currently, there is limited information regarding the prevalence of foodborne viruses in sea food in Iran, so the objective of the present study was to determine the prevalence rate of HAV in sea food samples obtained from the Isfahan and Shahrekord townships in Iran using a RT-PCR assay.

## MATERIAL AND METHODS

A total of 300 sea food samples were collected from September 2010 to April 2011 from supermarket and retail outlets in the Isfahan and Shahrekord townships in Iran. The sea food analysis was comprised of samples of fresh fish, crab, lobster and shrimp (Table 1). The samples were transferred to the Food Microbiology Laboratory at the Islamic Azad University of Shahrekord Branch in portable insulated cold-boxes. Samples were analyzed on the day they were collected.

Table 1. Prevalence of HAV isolated from sea food in Iran

| Fish (n=120) | Shrimp (n=120) | Crab (n=20) | Lobster (n=40) |
|---|---|---|---|
| Infected samples | Infected samples | Infected samples | Infected samples |
| 6(5%) | 2(1.66%) | 0(0%) | 0(0%) |

Two grams of inoculated mussel samples were rocked for 10min with 1mL of TRIzol® Reagent. The TRIzol® (Roche Applied Sciences) Reagent solution that reacted with the sample was kept separately. Another 1 mL TRIzol® Reagent was added to the sample rocked again for 10min and was brought together with the previously collected TRIzol® Reagent. The aqueous phase was taken after centrifugation (8000g, 20min, and $4^0$C) and stored by freezing. A volume of 100µL was purified by the use of an RNeasy Mini kit (Qiagen) according to the manufacturer's instructions (Baert *et al*., 2007).

Oligonucleotides were purchased from Cinnagen (Cinnagen, Iran). Sequences of oligonucleotides for amplifying a fragment of 267, respectively, from the HAV (Robertson *et al*., 1992; Normann *et al*., 1994) were as follows:
2949: 5'- TATTTGTCTGTCACAGAACAATCAG -3'
3192: 5'- AGG AGGTGGAAGCACTTCATTTGA -3'

cDNA synthesis was carried out using moloney murine leukemia virus reverse transcriptase (MMLV-RT, Fermentas) and random hexamer primers (Fermentas). Reverse transcription of heat-denatured RNA (5 min at 70°C in 32µL of reaction buffer for MMLV-RT in the presence of 0.1mM of each dATP, dCTP, dGTP and dTTP) was performed after the addition of 8µL of reaction mixture (10 mM dithiothreitol, 0.4µg of random hexamer, 5U of RNase inhibitor (Fermentas) and 400U of MMLV-RT) for 5min at 22°C, 15min at 37°C and 30min at 42°C. After reverse transcription, the reactions were heated to 99°C for 5min in order to inactivate MMLV-RT. Amplification of cDNA by PCR was carried out in a total volume of 50 µL in the reaction buffer for Taq DNA polymerase containing 1U of Taq DNA polymerase (Fermentas), 1µM of each primer (2949 and 3192), 1mM $MgCl_2$, 0.15mM dNTP, and 4µL of cDNA. Amplification was performed in 40 cycles of denaturation at 95°C for 30s, annealing at 60°C for 1min and extension at 72°C for 1min. After amplification, the PCR products were characterized through 1.5% agarose gel

electrophoresis in Tris–borate–EDTA buffer (Kingsley and Richards, 2001).

Statistical analysis: Data were analyzed using SPSS ver. 16.0 statistical software, a Chi-square test and fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $P<0.05$.

## RESULTS

According to results, 8 samples of total 300 studied samples were found to be infected with HAV. The product of 267bp was obtained, as expected, from RT-PCR amplification of the amplicon encoded portions of HAV genome. The number of infected samples and percentage of infection are shown in Table 1. The infection rate was lower for HAV as no infection was found in crab and lobster.

## DISCUSSION

A number of procedures have been reported for the detection of enteric viruses in sea food (Atmar *et al*., 1995; Cromeans *et al*., 1997; De Medici *et al*., 1998) and many of them have been applied to the study of viral contamination of shellfish from harvesting areas in different countries (Croci *et al*., 1999; Lee *et al*., 1999). Currently, there is limited information regarding the prevalence of enteric viruses in sea food in Iran. Therefore, the main purpose of the present study was to determine the prevalence rate of HAV in sea food samples obtained from the Shahrekord and Isfahan townships in Iran using a RT-PCR assay. In our study 6 out 120 (5%) fish, and 2 out 120 (1.7%) shrimp presented HAV. In the present study, no HAV isolate was detected in lobster and crab samples. To our knowledge, the present study is the first report of the detection of HAV in sea food in Iran; however, more research is needed to establish the prevalence rate of HAV in sea food.

The results of this study show that lobster and crab are not an important source for HAV infection in Iran. The sea food samples which were positive for HAV were collected from September to April. This result may indicate a potential point-source contamination.

The percentages of positive samples for HAV were similar to those obtained in other studies employing molecular detection procedures (Lee *et al*., 1999), however, higher contamination rates (25%-85%) have also been reported (Romalde *et al*., 2002; Kittigul *et al*., 2010). Also, in Italy, according to the Italian National Epidemiological Surveillance System for Acute Hepatitis Viruses (SEIEVA, Sistema Epidemiologico Integrato per le Epatiti Virali Acute'), in the period from 1995 to 1997, 71% of the noticed cases of acute viral hepatitis infection were cases of HAV infection. Variation in the prevalence of HAV isolates from sea food samples reported in other studies may be a result of different sampling techniques employed, seasonal effects and/or laboratory methodologies employed in different studies.

## CONCLUSIONS:

The presence of HAV in some sea food indicates the potential risk of infection with HAV in people consuming raw and uncooked sea food products. Therefore high-risk groups should avoid previously prepared uncooked sea food.

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